

Sex, Age and Breed Related Changes in Bovine Testosterone and Intramuscular Collagen

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SUMMARY

Longissimus muscle biopsies were obtained from forty bulls and steers representing Charolais, Simmental, Angus and Hereford breeds at 6, 9, 12, 15 and 18 months of age to evaluate the influence of age, breed and sex on the biosynthesis of muscle collagen. Blood samples were collected from each animal at each time period. The blood serum was harvested and assayed for concentrations of testosterone. Total collagen, soluble collagen and insoluble collagen were obtained on each sample. Soluble collagen and testosterone levels were significantly influenced by breed, sex and animal age. Total collagen levels were significantly affected by sex and age. Total collagen and testosterone levels peaked at 12 months of age. Breeds were ranked identically for total collagen and testosterone levels at 12 months. These data indicate that bulls are different from steers in synthesis of intramuscular collagen at or near 12 months of age. The increased synthesis of collagen appears to be influenced by testosterone or some other event associated with puberty. The mechanism of this action remains unclear.

INTRODUCTION

Castration of the male in meat-producing animals has long been a traditional practice in the production of commercial livestock. Numerous research studies have indicated that intact bovine males grow more rapidly, utilize feed more efficiently and produce a higher yielding carcass than castrates (Hedrick, 1969; Rhodes, 1969; Seideman *et al.*, 1982). Even

though young bulls have obvious growth and leanness advantages over steers, their meat is usually lower and more variable in tenderness than steers (Cross, 1982; Seideman *et al.*, 1982). These differences in tenderness have been attributed to differences in fatness (Riley *et al.*, 1983) or differences in connective tissue (Boccard *et al.*, 1979; Kopp, 1971; Crouse *et al.*, 1983).

Factors influencing the amount and strength of intramuscular collagen have been linked to animal age (Preston & Willis, 1970; Cross *et al.*, 1973), sex (Boccard *et al.*, 1979; Kopp, 1971), and breed (Andersen *et al.*, 1977; Liboriussen *et al.*, 1977). The literature strongly indicates that collagen solubility decreases significantly with animal age and that most of these changes take place from birth to about two years of age (Goll *et al.*, 1964b,c; Field *et al.*, 1966; Cross *et al.*, 1973; Kopp, 1971). Results by Field *et al.* (1966) and Kousgaard & Klastrup (1980) have illustrated that the age related changes in tenderness are significantly more pronounced in bulls than in steers and heifers, particularly in muscles high in collagen. These findings suggest that age related changes in the cross-linking of collagen might be related to sex of the animals.

Several workers (Vestergard, 1974; Liboriussen *et al.*, 1977; Kopp, 1971) reported an increase in collagen content in young bulls at about 12 months of age. Kopp (1971) suggested that the increase in collagen content at this age, which was accompanied by an increased solubility, was due to an increase in collagen synthesis related to the hormonal changes occurring during puberty in young bulls.

It has been observed that meat tenderness is influenced by genetics, both within and between cattle breeds (Preston & Willis, 1970; Andersen *et al.*, 1977; Koch *et al.*, 1982). To what extent these hereditary differences are due to connective tissue or myofibrillar proteins is not fully known.

The objective of this phase of our research is to investigate the influence of animal age, breed and sex condition (bull versus steer) on the content and solubility of intramuscular collagen using muscle biopsies in the *longissimus* muscle.

EXPERIMENTAL PROCEDURES

Selection and management of animals

Twenty bulls and twenty steers representing four breeds (7/8 Charolais, 7/8 Simmental, Hereford and Angus) were randomly selected for this

study. At five months of age the animals were placed on a ration of 78 % corn silage (IFN 3-08-153), 10 % corn (IFN 4-02-931) and 12 % supplement. Rations varied as the animals matured with the final ration being 42.7 % corn silage, 54.1 % corn and 3.2 % supplement.

Muscle and blood samples

Muscle biopsy samples (approximately 10 g) from the *longissimus* muscle of each animal were obtained at 6, 9, 12, 15 and 18 months of age. Sampling began in the posterior portion of the *longissimus* and continued an alternate sides to the 13th rib area. On the day of surgery, the animals were shaved and scrubbed with Septodyne serum. A local anesthetic (Epidural solution) was used to anesthetize the *longissimus*. Following surgery, Flo-Cillin was administered subcutaneously to prevent infections. *Longissimus* samples were trimmed of all epimysium, frozen in liquid nitrogen and stored at -70°C until analyzed for collagen. One week prior to each biopsy, blood samples were collected from each bull and steer. Serum was harvested from the blood samples and assayed for testosterone concentration (Schanbacher & D'Occhio, 1982).

Collagen analysis

Frozen powdered samples (4 g) were heated for 70 min at 77°C in one-fourth strength Ringer's solution and separated into supernatant and residue fractions following the procedure of Hill (1966). Each fraction was individually hydrolyzed in 6N HCl for 6 h at 1 atm pressure and 102°C . The hydroxyproline content was determined as outlined by Bergman & Loxley (1963). Collagen content (mg/g, fresh tissue basis) was computed by multiplying the hydroxyproline content of the insoluble portion by 7.25 (Goll *et al.*, 1964a) and that of the soluble portion by 7.52 (Cross *et al.*, 1973). The collagen content of the supernatant fraction expressed as a percentage of total collagen constituted percentage soluble collagen as specified by Hill (1966).

Statistical analysis

Data were analyzed by least-squares analysis of variance. The model was a split plot over time which included main effects for breed, age and sex and all interactions. Duncan's (1955) mean separation was used when main effects were significant at the $P < 0.10$ level of probability.

RESULTS

Results of the analysis of variance and subsequent *F* tests for collagen and testosterone traits are summarized in Table 1. The main effects for breed, sex and age were significant for soluble collagen and testosterone (sex not evaluated for testosterone). The main effect for breed was not significant for total or insoluble collagen, while sex and age were significant for all traits evaluated. The only significant interaction was the age by breed interaction for testosterone. Since the testosterone values for steers were consistently low (<0.5 ng/ml) over all age groups, these data were not included in the analysis.

TABLE 1
Analysis of Variance for Various Collagen and Testosterone Traits

<i>Sources of variation</i>	<i>Total collagen (mg/g)</i>	<i>Soluble collagen (%)</i>	<i>Testosterone (ng/ml)</i>
Breed	NS ^a	**	**
Sex	***	*	^b
Age	***	***	***
Breed × sex	NS	NS	
Age × breed	NS	NS	**
Age × sex	NS	NS	^b

* Significant at the $P < 0.10$ level of probability.

** Significant at the $P < 0.05$ level of probability.

*** Significant at the $P < 0.01$ level of probability.

^a NS = nonsignificant.

^b Analysis not conducted for sex effects since levels were low and biologically insignificant for steers.

$n = 200$ samples.

Breed effects

The influence of breed on collagen and testosterone levels is presented in Table 2. Even though total and insoluble collagen values were not significantly influenced by breed, soluble collagen and testosterone levels were. Percentage soluble collagen and testosterone were highest in the Simmental cattle, while testosterone was lowest in the Hereford cattle. Similar results by Liboriussen *et al.* (1977) reported significant differences in soluble collagen between sire breeds. These authors reported that

TABLE 2
The Influence of Breed on Mean Collagen and Testosterone Traits

Breed	n	Total collagen (mg/g)		Soluble collagen (%)		Testosterone (ng/ml)	
		Mean	SE	Mean	SE	Mean	SE
Simmental	10	4.97 ^a	0.34	16.99 ^a	0.65	5.21 ^a	0.45
Charolais	10	5.73 ^a	0.41	14.87 ^b	0.77	4.31 ^b	0.52
Hereford	10	5.24 ^a	0.36	15.06 ^b	0.69	2.90 ^c	0.44
Angus	10	5.66 ^a	0.36	14.43 ^b	0.68	4.97 ^a	0.45

^{a-c} Means in the same column with different superscripts are significantly different ($P < 0.05$).

earlier maturing breeds (e.g. Hereford) had less soluble collagen than later maturing breeds (e.g. Charolais) when comparisons were made at the same chronological age. Boccard *et al.* (1979) also found differences in the content and solubility of collagen between cattle breeds (Afrikaaner and Friesian).

Sex effects

Sex (bull versus steer) had significant effects on all collagen traits (Table 3). When age and breeds were combined, the *longissimus* from bulls contained more soluble collagen and less total collagen. The magnitudes of the differences presented in Table 3 are not large, but are significant.

TABLE 3
The Influence of Sex on Mean Collagen Content in the *Longissimus*

Sex	Total collagen (mg/g)		Soluble collagen (%)	
	Mean	SE	Mean	SE
Bull	5.75 ^a	0.26	15.92 ^b	0.49
Steer	5.05	0.26	14.76	0.49

^a Means in the same column are significantly different at the $P < 0.10$ level of probability.

^b Means in the same column are significantly different at the $P < 0.01$ level of probability.

TABLE 4
The Influence of Age on Mean Collagen and Testosterone Traits
(Bulls and Steers Combined)

Animal age (months)	Total collagen (mg/g)		Soluble collagen (%)		Testosterone (ng/ml)	
	Mean	SE	Mean	SE	Mean	SE
6	3.89 ^a	0.38	19.26 ^c	0.73	2.22 ^b	0.43
9	4.74 ^b	0.38	20.73 ^c	0.73	2.95 ^d	0.43
12	8.91 ^c	0.39	14.83 ^d	0.75	9.09 ^e	0.44
15	5.39 ^d	0.46	11.94 ^b	0.87	3.13 ^d	0.57
18	4.06 ^a	0.44	9.93 ^a	0.83	^e	

^{a-d} Means in the same column with different superscripts are significantly different ($P < 0.05$).

^e Analysis not conducted for this age group.

Age effects

The influence of age on changes in collagen content and solubility have been studied by numerous scientists (Goll *et al.*, 1964^{b,c}; Cross *et al.*, 1973; Kopp, 1971; Boccard *et al.*, 1979). These workers, and many others, agree that collagen solubility decreases significantly with the age of the animal. Data reported in Table 4 would support these conclusions. Also of interest in Table 4 is the relationship between total collagen and testosterone. Total collagen increased up to 12 months of age and then decreased significantly. The same trend was apparent for testosterone (Table 5).

TABLE 5
Age by Breed Interaction Means for Testosterone Levels in the Bull

Animal age (months)	Serum testosterone levels (ng/ml)							
	Simmental		Charolais		Hereford		Angus	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
6	2.71	0.85	1.29	0.85	1.31	0.86	3.58	0.85
9	3.10	0.85	3.23	0.86	1.82	0.85	3.63	0.84
12	9.11	0.84	10.09	0.85	7.06	0.85	10.13	0.85
15	5.92	0.98	2.63	1.48	1.43	0.98	2.54	0.97

TABLE 6
Age by Sex Interaction Means for Collagen Traits in the *Longissimus*

Animal age (months)	Soluble collagen (%)				Total collagen (mg/g)			
	Bull		Steer		Bull		Steer	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
6	19.63	1.03	18.88	1.03	4.03	0.54	3.75	0.54
9	21.61	1.03	19.86	1.02	4.89	0.54	4.58	0.53
12	16.74	1.02	12.91	1.09	9.99	0.53	7.83	0.57
15	11.58	1.26	12.30	1.16	5.36	0.66	5.42	0.61
18	10.04	1.18	9.83	1.11	4.49	0.62	3.64	0.59

Age/breed interactions

Interaction means for testosterone levels are presented in Table 5. The increase to 12 months followed by a decrease is evident across all breeds. The highest levels at 12 months are in the Angus and Charolais breeds while the lowest is in the Hereford breed. The total collagen at 12 months ranks the breeds in the same order as does testosterone at 12 months.

Age/sex relationships

Even though the age/sex interaction was not significant, the means are presented in Table 6 to give a clearer picture of the sex/age relationship. Total collagen increased to 12 months and then decreased in both bulls and steers. The obvious question is, why the increase in collagen in steers, when the testosterone levels were not affected by age? As expected, the soluble collagen decreased as age increased. The magnitude of the decrease was much less in bulls, particularly at 12 months. This could perhaps indicate some endocrine influence above and beyond the influence of testosterone.

DISCUSSION

Numerous studies have compared the palatability traits of bulls versus steers and the majority indicate lower tenderness ratings for bulls versus

steers regardless of age (Cross, 1982; Seideman *et al.*, 1982). Very little work has been reported as to the cause of these tenderness differences. Some of the differences could be related to cold toughening effects and could perhaps be alleviated by post mortem electrical stimulation (Riley *et al.*, 1982). Crouse *et al.* (1983) found no tenderness benefit from electrical stimulation but did find improvement from high temperature conditioning. It is likely that toughness in young bulls is caused by a combination of factors.

These data add further support to the age effects on collagen cross-linking and collagen solubility. Results also reveal an interesting relationship between collagen synthesis and possible endocrine influences. These differences also appear to be influenced by breed. Other workers have reported an increase in intramuscular collagen content in bulls at 12 months of age (Vestergard, 1974; Kopp, 1971). It appears from the present and other investigations, that for bulls the collagen content increases near puberty.

The increased collagen synthesis near puberty would result in an increase in the proportion of immature collagen, less cross-linking and thus a greater proportion of collagen that would be solubilized during cooking. Since these bulls would probably be marketed at a later age (14 to 16 months), the cross-linking would be expected to continue, and the total amount of cross-linked (toughened) collagen would also be higher in bulls. The impact of this increase on tenderness will require further study, but one could hypothesize that this situation could be a significant contributor to the toughness in bulls.

In conclusion, the data from this study indicate that bulls are different from steers in regard to relative synthesis of intramuscular collagen at or near puberty. The increase synthesis of collagen appears to be influenced by testosterone or some related endocrine parameter. The mechanism of this action remains unclear.

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